Shared epitope is associated with reactivity of Th17 cells to cigarette smoke extract regardless of smoking history.

Running title: Shared epitope and Th17 reactivity.

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Rheumatoid arthritis (RA) severity has been linked to combination of the HLA-DRB1 amino acid sequence motif called “shared epitope” (SE) and cigarette smoking (CS) (1). Animal studies support the association of DRB1 alleles and CS with arthritis susceptibility (2, 3) and highlight the involvement of IL-17 producing T helper cells (Th17) in the disease through the activation of the aryl hydrocarbon receptor (AhR). We have previously reported (4) on a limited group of donors that cigarette smoke extract (CSE) treatment of Th17 cells leads to reduced cytokine production and prevents normal differentiation of human Th17. Taking into consideration the known association between smoking and genotype in RA we performed a pilot study in which we enrolled healthy individuals who reported themselves as active smokers (table 1) and evaluated possible association between smoking status, genetic backgrounds and reactivity of Th17 cells to CSE.

Study group included anonymized healthy blood donors that reported themselves as active smokers (n=22) and control non-smokers (n=36) (supplementary table 1). Informed consent from all subjects was obtained prior to blood donation by the Oslo Blood bank according to the Norwegian laws and regulations (approval 2015/1591, Norwegian South-Eastern REC). Th17 cells were cultured as described previously (4) in presence of 21% or 1% oxygen. The cytokines in supernatants were analysed using a Legendplex kit (Biolegend) on a BD Fortessa flow cytometer (Oslo University flow cytometry Core facility). Genotyping was done using SBT Resolver DRB1 kit (Conexo Genomics, Australia), data analysed with Assign Software (Conexio Genomics).

Similar to our previous study, CSE treatment resulted in overall reduced cytokine production, however, this was not the case for Th17 cells from SE+ donors where significant increase of IL-17A production was observed (figure 1A). Further, Th17 cells from SE+ donors showed a trend to produce more IL-17A under additional 1% oxygen treatment counteracting the negative effect of physiological hypoxia on the cytokine production (figure 1B). Interestingly, the observed effects were independent and not correlating to other donor’s features such as age, sex, blood type and smoking history. We would like to stress that unlike animal studies showing link between AhR stimulation and Th17 (3) that use purified AhR ligands we used whole CSE preparation that contains hundreds of chemicals and as we believe mimics the effects of cigarette smoking better. CSE affects not only AhR but also other signalling pathways and transcriptional factors, e.g. by reducing ROCK2-dependent phosphorylation of Interferon regulatory factor 4 (IRF4) (4, 5) leading to alterations in T cell function.

Despite a limited pilot study, we document reactivity to environmental toxins by Th17 that is linked to a genotype of healthy persons prior to disease. Unlike large RA studies we were not able to link smoking history of the individual and SE+. We speculate that this may in part be due to a smaller cohort examined by us. However, it may also be due to physiological factors involved in e.g. metabolism of cigarette smoke chemicals. Chemicals in cigarette smoke inhaled through the lungs would first be metabolized and processed through the cytochrome P450 or other phase II drug metabolizing enzymes expressed in lung tissue(6) and then target a number of different tissues including circulating immune cells, cartilage and the bones.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Figure 1. IL-17A production is reduced by CSE treatment and low oxygen, SE is associated with sensitization to CSE regardless of smoking history. A. Th17 cells treated with CSE from SE+ subjects produce more IL-17A (p=0,048), regardless of smoking history. **B.** low oxygen treatment reduced IL-17A production regardless of SE or CSE treatment (p<0,001). The statistical significance of univariate associations between IL-17A levels and DRB1 shared epitopes, smoking status, CSE treatment, and oxygen conditions was assessed by Student's t-tests. Multivariate associations were assessed by ANOVA models. Two-sided p-values < 0.05 are considered to indicate statistically significance. All statistical analyses were performed in R 3.5.0(7) and graphs were prepared in GraphPad Prism v5.04.